**TITLE PAGE**

**Title:** Germline BRCA mutation and outcome in young onset breast cancer: POSH, a prospective cohort study.

**Author names and affiliations:** Ellen R Copson PhD*[1], Tom C Maishman MSc*[2], Will J Tapper PhD[3], Ramsey I Cutress PhD[1], Stephanie Greville-Heygate MB ChB[1,4], Prof Douglas G Altman PhD[5], Bryony Eccles MD[1], Sue Gerty BSc[2], Lorraine T Durcan BSc[2], Prof Louise Jones PhD[6], Prof D Gareth Evans MD[7], Prof Alastair M. Thompson MD[8], Prof Paul Pharoah PhD[9], Prof Douglas F Easton PhD[9], Alison M Dunning PhD[9], Prof Andrew Hanby BM[10], Prof Sunil Lakhani PhD[11], Prof Ros Eeles PhD[12], Prof Fiona J Gilbert MB BCh[13], Hisham Hamed MD[14] , Prof Shirley Hodgson DM[15], Peter Simmonds MB BS[4], Louise Stanton MSc[2], Prof Diana M Eccles MD[1,4~]

[*] *Joint first authors ~ Corresponding author*

[1]Cancer Sciences Academic Unit, Faculty of Medicine, University of Southampton, Southampton SO16 6YD, UK.

[2]Southampton Clinical Trials Unit, University of Southampton, Southampton SO16 6YD, UK

[3]Genetic Epidemiology and Genomic Informatics Group, Human Genetics, Faculty of Medicine, University of Southampton, Southampton SO16 6YD, UK.

[4]University Hospital Southampton NHS Foundation Trust, Southampton SO16 6YD, UK.

[5] Centre for Statistics in Medicine, Nuffield Department of Orthopaedics, Rheumatology & Musculoskeletal Sciences, University of Oxford, Oxford OX3 7LD, UK.

[6]Tumour Biology Department, Institute of Cancer, Barts & The London School of Medicine & Dentistry, Charterhouse Square, London, EC1M 6BQ, UK.

[7]Centre for Genomic Medicine, Division of Evolution and Genomic Sciences, University of Manchester MAHSC, St Mary's Hospital, Oxford Road, Manchester M13 9WL, UK.

[8] University of Texas M.D. Anderson Cancer Center, Houston, Texas, 77030, USA.

[9]Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge CB1 8RN, UK.

[10]Department of Pathology, University of Leeds, Faculty of Medicine, Leeds LS2 9JT.

[11]Discipline of Molecular & Cellular Pathology, Faculty of Medicine, University of Queensland, The Royal Brisbane & Women's Hospital, Herston 4029, Brisbane, Australia.

[12]Institute of Cancer Research, 123 Old Brompton Road, London SW7 3RP, UK.

[13]Department of Radiology, University of Cambridge, Cambridge Biomedical Campus, Hills Road, Cambridge CB2 0QQ, UK.

[14]Guy's & St Thomas' Hospital, Great Maze Pond, London, SE1 9RT, UK.

[15]St. George's Hospital, University of London, London SW17 0QT, UK.

Contact information for corresponding author: Prof Diana Eccles. Email: D.M.Eccles@soton.ac.uk. Address: Cancer Sciences Academic Unit, Faculty of Medicine, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton, SO16 6YD, United Kingdom.

ABSTRACT

**Background**

Retrospective studies provide conflicting interpretations of the effect of inherited genetic factors on breast cancer prognosis. The primary aim of this study was to determine the impact of a germline BRCA1/2 mutation on outcomes in young onset breast cancer.

**Method**

Patients were recruited from 127 UK oncology centres and were eligible if aged $\leqslant$ forty years at first diagnosis of invasive breast cancer. BRCA1/2 mutations were identified using blood DNA collected at recruitment. Clinicopathological, treatment and long term outcome data were collected from routine medical records. The primary outcome was overall survival (OS) of all BRCA1/2 carriers vs. all non-carriers, assessed using Cox proportional-hazards models, or flexible parametric survival models (FPSMs) for models which involved time-varying hazards. Recruitment was completed in 2008; long term follow-up continues.

**Findings**

Between 2000-2008, 2733 women were recruited. Genotyping detected a pathogenic mutation in 337 (12·3%) of 2733 patients (201 BRCA1, 136 BRCA2). At a median follow-up of 8·2 years, (inter-quartile range: 6·0 to 9·9 years), 651 (96%) of 678 deaths were due to breast cancer. There was no significant difference in OS between BRCA1/2 mutation carriers and non-carriers in multivariable analyses (OS: HR 0·96; 95% CI 0·76-1·22; p=0·76). However, in patients with triple negative breast cancer, (n=558), BRCA mutation carriers showed a different pattern of relapse over time compared to non-carriers and significantly better OS at two years, (HR 0.59 [95% CI 0·35-0·99], p=0·047).

**Interpretation**

Young onset breast cancer patients have a high mortality and those who carry a BRCA gene mutation have similar survival to non-carriers. *BRCA* carriers presenting with triple negative breast cancer may

have a survival advantage during the first few years after diagnosis compared to non-carriers. Decisions about timing of additional surgery aimed at reducing future second primary cancer risks should take into account prognosis associated with the first malignancy and patient preference.

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**Research in Context**

**Evidence before this study**

At the initiation of this cohort study (December 1999), a small number of retrospective studies reporting prognosis in *BRCA* gene carriers had been published. In December 2016, we performed another PubMed search for studies of BRCA1/2 mutations carriers and prognosis, using the following search terms: '(BRCA) AND (survival or prognosis or outcome or mortality) AND (breast neoplasms or breast neoplasm or breast cancer or breast tumour)'. Our search was not limited by date or language. References cited in review papers were hand searched for additional papers. Previous studies and more recent meta-analyses have reported inconsistent effects of BRCA1 and 2 mutations on the outcomes of early breast cancer with better, worse and the same outcomes of *BRCA*1/2 mutation carriers compared to sporadic breast cancer cases. These conflicting results may be explained by methodological issues with ascertainment biases introduced by retrospective and selective identification of cases, incomplete genetic testing, small numbers, lack of adjustment for clinical variables, including treatment, and limited follow-up.

**Added value of this study**

POSH is the largest prospective cohort study to compare breast cancer outcomes of BRCA1/2 mutation carriers with sporadic cases and is strengthened by unbiased recruitment, universal and central genetic testing at the end of the study and comprehensive pathological, clinical and follow-up data.

**Implications of all the available evidence**

The overall survival of early breast cancer patients with BRCA1/2 mutations is not significantly different to that of patients with sporadic breast tumours when tumour and treatment factors that affect prognosis are taken into account. However within the triple negative (estrogen receptor negative, progesterone receptor negative and Her-2 receptor negative) breast cancer subgroup,

**BACKGROUND**

Although only 5% of breast cancers are diagnosed in women aged less than 40 years, this age group experiences a high proportion of breast cancer deaths and includes a higher proportion who carry a

pathogenic *BRCA*1 or *BRCA*2 gene compared to older onset.[1-3] Second primary breast cancers are more frequent in high risk gene carriers and this drives early genetic testing to inform surgical decision making; however it is currently unclear whether a germline *BRCA*1/2 mutation has independent prognostic implications after initial cancer diagnosis.

*BRCA*1 loss of function is associated with high grade, ER (oestrogen receptor)/ PR (progesterone receptor)/ HER 2 (human epidermal growth factor receptor 2) negative ("triple negative") breast cancer (TNBC) with a basal-like gene expression profile.[4] *BRCA*2 associated breast tumours are usually high grade ER positive and HER2 negative tumours.[5, 6] *BRCA*1 carriers have been reported to have enhanced sensitivity to neoadjuvant chemotherapy using cytotoxic drugs.[7]

Published studies and meta-analyses have reported better, worse and the same outcomes of *BRCA*1/2 mutation carriers compared to sporadic breast cancer cases.[8-14] A recent comprehensive meta-analysis of 66 studies of breast cancer survival in *BRCA*1/2 carriers compared to non-carriers or the general breast cancer population, which assessed study quality as well as outcome data, concluded that "it is not yet possible to draw evidence based conclusions about the association between *BRCA*1 and/ or *BRCA*2 mutation carriership and breast cancer prognosis".[12]

The primary aim of this study, Prospective Outcomes in Sporadic *versus* Hereditary breast cancer (POSH), was to determine the impact of inherited BRCA1/ 2 mutations on outcomes in young onset breast cancer.[15, 16]

## METHODS

### Study Design and Participants

POSH recruited young women (aged 18 to 40) diagnosed with primary breast cancer in the United Kingdom between 2000 and 2008, (http://www.southampton.ac.uk/medicine/research/posh.page). The protocol was published in 2007.[15]

Patients recruited from 127 UK hospitals were eligible if diagnosed with invasive breast cancer aged 40 years or younger (Appendix Table 1 pp 1-2 lists recruiting sites); the earliest date of diagnosis was 24th January 2000 and latest diagnosis 24th January 2008. Potential recruits were identified within 12 months of initial diagnosis of invasive breast cancer and date of diagnosis was defined as the first histological confirmation of invasive breast cancer and median time from diagnosis to blood draw was 5·5 months. All histological subtypes, disease stages (I-IV), co-morbidities and performances status were permitted. Patients with a previous invasive malignancy (with the exception of non-melanomatous skin cancer) were excluded. Written informed consent was obtained from all participants. Ethical approval was granted in 2000 (MREC 00/6/69) and the study was approved for recruitment as part of the UK National Cancer Research Network (NCRN) portfolio in 2002, subsequently the NIHR portfolio.

**Procedures**

All patients received treatment according to local protocols. Details of personal characteristics, tumour pathology, disease stage, and surgical and cytotoxic treatment data were collected from medical records at study entry. Family history was collected by questionnaire. The BOADICEA algorithm [http://ccge.medschl.cam.ac.uk/boadicea/], without adjustment for pathological subtype, was used to estimate the probability that an individual might carry a *BRCA*1/2 pathogenic variant.[17] Pathology and imaging data were verified with copies of original reports from sites. For patients treated with neoadjuvant chemotherapy, initial tumour diameter was derived from radiological reports.

ER, PR and HER2 receptor status of primary tumours was determined from reports of local routine pathology testing of diagnostic core biopsies or tumour resections for clinical use. Hormone receptor levels equivalent to an Allred score of $\geqslant$3 were categorised as positive. Immunohistochemical

staining of tissue microarrays (TMAs) in 1336 cases, during 2012 and 2016, allowed clinical source data for ER, PR and HER2 receptor status to be corroborated; TMA scores were used to supplement missing data points for these receptors.[16]

DNA for genotyping was extracted from whole blood samples submitted at recruitment. A multiplex amplicon based library preparation system, Fluidigm Access Array™ (Fluidigm UK Ltd, Cambridge, UK), targeted a panel of breast cancer susceptibility genes (including *BRCA1/2* and *TP53*) for massively parallel sequencing using an Illumina HiSeq2500 Next Generation Sequencing Platform Illumina Inc. UK, Little Chesterford, UK), details are provided in Appendix Methods 1 (pp 20-21). Targeted sequence capture cannot reliably identify large exonic deletions or duplications so multiplex ligation probe analysis (MLPA) was used in patients who met current guideline thresholds for clinical genetic testing.[17, 18] Predicted protein truncating variants (frameshift, nonsense and canonical splice site and large rearrangements) plus other (mainly missense) variants unequivocally defined as pathogenic based on multiple lines of evidence and expert review were assigned to the BRCA mutation carrier group (BRCA+). All pathogenic variants were confirmed by Sanger sequencing. All other patients, including those carrying *BRCA*1/2 variants of uncertain significance or very low penetrance, were assigned to the same group as no mutation found (*BRCA*-) or excluded if found to carry a pathogenic variant in *TP53*. For the purposes of this analysis, mutations in other breast cancer genes were not curated.

The study protocol and patient information specified that patients would not be informed of the research genetic testing results. Patient information sheets gave information about seeking clinical genetic referral. Clinical referrals for genetic testing were made by the treating physician according to local protocols. Genetic test reports for study patients generated by NHS diagnostic laboratories were collected as part of the medical record.

Detailed clinical follow-up data, including date and site of disease recurrence, were obtained from medical records at 6, 12 months and annually thereafter until death or loss to follow-up. Patients were flagged in the National Health Service Medical Research Information Service for automatic notification of date and cause of death. We included all data received until 26 July 2016.

**Outcomes**

The primary outcome was overall survival (OS) defined as time from first diagnosis to death from any cause. Additional study outcomes included distant disease-free survival (DDFS) defined as time from first diagnosis to first distant disease, excluding local (in breast) recurrence.

**Statistical Analysis**

The original study sample size of a minimum of 2000 was estimated based on a prevalence of *BRCA*1/2 pathogenic mutations of 10%, and an absolute difference in two-year event rate between carriers and non-carriers of 10% (20% in gene carriers compared with 10% in sporadic cases).[15] We also considered a BRCA1/2 mutation prevalence of 5% and 15% and larger sample sizes. Good recruitment rates and data returns allowed us to continue study recruitment beyond 2000 (actual sample size 3021) providing sufficient power for multivariable analyses (MVA).

Statistical analyses were conducted according to a pre-specified plan (Appendix Documents 1 and 2, pp 22-31).[19] The analysis population was made up of all eligible patients recruited to the cohort who had primary tumour and genotyping data available, were aged ≤40 years at the date of diagnosis, did not carry a TP53 gene, and who did not present with metastatic disease at presentation (M1 stage). An important pre-specified subgroup of the analysis population was patients with triple-negative breast cancer (TNBC population). All analyses were carried out on both the analysis population and TNBC population, unless specified otherwise. Key patient data were described by *BRCA* status, and formal comparisons by *BRCA* status were carried out using Mann-Whitney tests (for continuous variables) and Pearson $\chi^2$-tests (for categorical variables), and were carried out on patients with

complete data. Kaplan-Meier plots were used to describe survival data by *BRCA* status at two, five and ten years. A two year comparison was chosen as this time-point was specified in the original sample size, five and ten year comparisons are commonly used and clinically relevant time points. Patients who did not experience an event were censored at the date of last follow-up. Hazard ratios (HRs) and 95% confidence intervals (CIs) for univariable analyses (UVA) and MVA (for the primary and secondary outcomes) were calculated using Cox proportional-hazards models, or flexible parametric survival models (FPSMs) for models which involved time-varying hazards.[20] For each FPSM, varying degrees of freedom for the baseline-hazard rate and time-dependent effect were explored to obtain the best model fit. All missing data were assumed to be either missing at random or missing completely at random, and censoring assumed to be non-informative. All analyses were performed using Stata, version 14·2, (StataCorp, College Station, TX, USA), and multiple-imputation was incorporated in the MVA, generated using the mi command. Pre-specified sensitivity analyses included generating corresponding complete-case MVA model results. In addition, to investigate the degree of potential bias from time of diagnosis to registration blood draw, a MVA model adjusting for the time from diagnosis to blood draw was generated accordingly (TNBC population only).

**Role of the funding source**

The funders and their representatives had no role in the study design, data collection, analysis or interpretation of results or in the writing of this manuscript and the decision to submit it for publication. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

## RESULTS

### Genotyping.

Overall 337 (12·3%) of 2733 patients included in the analysis population of the cohort had either *BRCA*1 (201) or *BRCA*2 (136) mutations, of which 44 (12·6%) patients had large copy number variants

(Appendix Table 2, pp 3-7). Clinical *BRCA*1/*BRCA*2 mutation testing had occurred in 388 (14·2%) of the analysis population, of which 182 (46·9%) had pathogenic mutations.

**Patient characteristics**

The study recruited 3021 eligible patients, of whom 2733 (90·5%) were included in the analysis population, and 288 (9·5%) were excluded (M1 stage [74]; no clinical data [2]; no genotyping data [160], gene carriers aged 41-50 [42], or *TP53* mutation carrier [10]) (Appendix Figure 1, pp 11). Median time from breast cancer diagnosis to study registration blood draw was 5·5 months (inter-quartile range: 3·2-10·7 months). There were several significant clinicopathological differences between *BRCA*1 and *BRCA*2 carriers and non-carriers (Table 1). Carriers had higher tumour grade and fewer HER2 over-amplified tumours than non-carriers. A higher proportion of *BRCA*1+ patients received breast-conserving surgery (n=106, 52·7%) compared to *BRCA*2+ (n=43, 31·4%) and *BRCA*- patients (n=1188, 49·6%). The most commonly used chemotherapy regimen included anthracycline +/- taxanes.

Of the 2733 patients in the analysis population, 558 (20·4%) presented with TNBC (TNBC population). BRCA mutations were identified in 136 (24·4%) of the TNBC patients, of which 123 (90·4%) were *BRCA*1. Differences in tumour characteristics between *BRCA*1 and *BRCA*2 were also seen in TNBCs (Table 2).

**Follow-up and Survival**

Median follow-up was 8·2 years, (inter-quartile range: 6·0 to 9·9 years); 91 (3·3%) of the analysis population were lost to follow-up. Contralateral breast tumours occurred in 151 (5·5%) patients overall; 37 (18·4%) in *BRCA*1 carriers (n=201), 17 (12·4%) in *BRCA*2 carriers (n=137) and 97 (4·1%) in non-carriers (n=2395). Median time to contralateral breast cancer was 3·0 years, (range: 0·02 to 9·3 years) in *BRCA* mutation carriers and 2·7 years, (range: 0 to 11·5 years) in non-carriers. Seven-hundred and fifty-two (27·5%) women developed a distant recurrence. Of the 678 deaths observed,

651 (96·0%) were due to breast cancer. Deaths due to non-breast malignancies, (Appendix Table 3, pp 8) included 6 (3%) new primary cancers in *BRCA*1 mutation carriers, (3 ovarian, 1 primary peritoneal, 1 oesophageal and 1 pancreatic) and 12 (0·5%) malignancies in non-*BRCA* mutation carriers, (4 haematological, 3 lung cancers and one each of brain, colorectal, gastric, pancreatic, sarcoma). There were no deaths attributed to second primary cancers amongst *BRCA*2 mutation carriers.

OS (95% CI) in *BRCA*+ and *BRCA*- patients respectively was 97·0% (94·5% to 98·4%) versus 96·6% (95·8% to 97·3%) at two-years; 83·8% (79·3% to 87·5%) versus 85·0% (83·5% to 86·4%) at five-years; and 73·4% (67·4% to 78·5%) versus 70·1% (67·7% to 72·3%) at ten-years (Figure 1). There was no statistical difference even after adjusting for known prognostic factors (UVA and MVA HR [95% CI, p-value]: 0·99 [0·78-1·24, 0·90] and 0·96 [0·76-1·22, 0·76], respectively). Similar results were found when comparing DDFS by *BRCA*+/- status (Appendix Figure 2, pp 12). Comparison of *BRCA*- with *BRCA*1+ or *BRCA*2+ for OS separately gave similar results (Appendix Figure 3 and 4, pp 13-14).

In the TNBC sub-population, 159 (28·5%) women developed a distant recurrence, 153 (27·4%) died and all deaths were due to breast cancer. The estimated hazard for relapse after TNBC diagnosis varied over time (Figure 2). OS was significantly better for *BRCA*+ compared to *BRCA*- at two-years, 94·8% (95% CI: 89·4% to 97·5%) vs. 91·4% (95% CI: 88·2% to 93·7%) (MVA FPSM HR [95% CI, p-value]: 0·59 [0·35-0·99, 0·047]); the absolute difference was greatest at five-years, 81·3% (95% CI: 73·4% to 87·1%) vs. 74·2% (95% CI: 69·7% to 78·2%) (MVA FPSM HR [95% CI, p-value]: 1·13 [0·70-1·84, 0·62]); and least at ten-years 72·1% (95% CI: 61·9% to 79·9%) vs. 68·8% (95% CI: 63·4% to 73·6%), (MVA FPSM HR [95% CI, p-value]: 2·12 [0·82-5·49, 0·12]), (Figure 3). For DDFS however the difference was not significant, (Appendix Figure 5, pp 15). Inclusion of time from diagnosis to registration blood draw in MVA did not affect results, (Appendix Figure 6, pp 16). For all analyses, results with imputation were almost identical to complete case results (Appendix Tables 4 and 5, pp 9-10). Results from tests of proportional hazards are presented in Appendix Figure 7, pp 17.

Further analyses, (not included in the prior statistical analysis plan), were conducted to explore the possible reasons for some of our results in the TNBC group. We considered if the superior outcome of *BRCA* mutation carriers with TNBC could be due to a beneficial effect of risk reducing surgery in *BRCA* carriers so we repeated the TNBC analysis excluding 31 (5·6%) of the TNBC population (21 BRCA+ and 10 BRCA-) who underwent bilateral mastectomy within the first year following diagnosis. This sensitivity MVA showed a significant difference in OS at 2 years (2-year OS% (95% CI) BRCA+ vs. BRCA-: 94·7% [88·6% to 97·6%] vs. 91·2% [88·0% to 93·5%]; HR [95% CI], p: 0·52 [0·29 to 0·.91], p=0·023). There was a slight increase in absolute survival difference for *BRCA* carriers at 5 years (from 7·1% to 8·8%), although the MVA result was not significant (5-year OS% (95% CI) BRCA+ vs. BRCA-: 82·6% [74·1% to 88·6%] vs. 73·9% [69·2% to 77·9%]; HR [95% CI], p: 0.98 [0·58 to 1·65], p=0·94) (Appendix Figure 8, pp 18). A further sensitivity analysis was undertaken to explore the observed pattern of improved survival at an early time point with apparently worse survival in the long term. We repeated the primary analysis in TNBC patients excluding 37 (6·6%) of patients who developed a new primary breast or ovarian cancer. This analysis showed that the OS HR at 10 years for *BRCA*1/2 mutation carriers versus non-carriers fell from 2·12 to 1·24 ([95% CI], p: [0·39 to 3·96], p=0·73; 10-year OS% (95% CI) BRCA+ vs. BRCA-: 78·4% [69·0% to 85·3%] vs. 69·3% [63·7% to 74·2%]) (Appendix Figure 9, pp 19).

**DISCUSSION**

Following a diagnosis of early breast cancer, *BRCA* mutation carriers are frequently offered additional management options including bilateral mastectomy. Any prognostic implication of carrying a *BRCA* mutation for primary treatment is important to clarify to facilitate clinician and patient decisions around the optimum timing of additional surgery. Furthermore, clinical trials of treatment specifically targeted toward *BRCA* gene carriers may need to take into account any effect of *BRCA* mutational status on primary treatment outcomes. We found no significant difference in OS

or DDFS between *BRCA*1/2 mutation carriers and non-carriers after breast cancer diagnosis in unadjusted or adjusted analyses including adjustment for ethnicity and body mass index.[21, 22]

To our knowledge, this is the largest prospective study to report the prognostic implication of germline *BRCA* mutations and the only one with a pre-planned analysis in patients presenting with triple negative tumours. Breast cancer mortality was high; only 2% of patients were in a screening programme, seven patients had been identified as *BRCA* carriers prior to their diagnosis, 3/7 were diagnosed during surveillance imaging. Our results are in broad agreement with some previous studies,[8-10, 23] but others have reported conflicting results.[24, 25,26] Ascertainment biases introduced by retrospective and selective identification of cases, incomplete genetic testing, small numbers, lack of adjustment for clinical variables including treatment and limited follow-up likely explain many discrepancies although recent studies have generally used stronger methodology.[11, 12]

The percentage of *BRCA*+ patients in the POSH cohort (12·4%) was higher than anticipated from historical studies of cases diagnosed ≤40 years, perhaps due to more sensitive mutation testing options.[1] Twenty-two per cent of gene carriers (75/338) did not meet current family history or pathology based genetic testing guidelines.[18] Only 14% of all patients had clinical genetic testing. The ratio of *BRCA*1 to *BRCA*2 mutations was 1·5:1 which is similar to that reported in other large Western population based cohorts. [2, 23] Deaths due to other malignancies were low in frequency in all groups reflecting the young age group; however, *BRCA*1 deaths included potentially preventable ovarian cancers at ages 41-46 years. Bilateral risk reducing mastectomy is not a necessary part of treating a unilateral breast cancer but unilateral mastectomy may enable breast radiotherapy to be omitted. Discussion about future primary cancer prevention during primary breast cancer treatment must take into account individual circumstances including likely tumour prognosis and the physical and psychological implications of more extensive surgery. In this cohort **immediate bilateral** mastectomy was not associated with improved survival although the reported use of risk reducing surgery in this cohort was low**;** bilateral salpingo-oophorectomy (BSO) was recorded in 32 patients

and bilateral mastectomies in 107 patients.[27] This likely reflects the low level of clinical testing at the time of the study. Although risk reducing BSO is clearly highly effective at reducing ovarian cancer incidence, however the risk of primary peritoneal cancer is not reduced and recent studies indicate the previously reported effect of this procedure on future breast cancer risk in *BRCA*1 and *BRCA*2 mutation carriers may have been overestimated because of uncorrected bias.[28]

Our analysis of the 558 TNBC patients in this cohort shows an intriguing difference in the hazard for overall survival over the first few years after diagnosis. *BRCA* gene carriers were less likely to experience early breast cancer death than non-carriers. This early survival advantage is also observed amongst ovarian cancer *BRCA* carriers.[29, 30] If real, this may reflect greater sensitivity of breast cancers in *BRCA* carriers to chemotherapy or the greater visibility of *BRCA* carrier cancers to host immune attack.[31] One theory that could explain the slight survival advantage for BRCA carriers not undergoing immediate bilateral mastectomy, is that a major surgical intervention may compromise host immunity at a time when this is particularly important for eradicating micrometastases. This hypothesis would need further exploration due to the reduced numbers within this subgroup.

Several publications have suggested that the DNA repair deficiency associated with *BRCA* mutations results in enhanced sensitivity to many chemotherapy agents, in particular higher response rates to platinums have been observed in both metastatic and neo-adjuvant settings.[4,7] Only thirteen patients in this cohort were treated with platinum based adjuvant regimens for early breast cancer, including one *BRCA*1 and one *BRCA*2 carrier.

Our study illustrates the high breast cancer mortality in this unscreened young population and the impact of known tumour and patient prognostic characteristics. Inevitably, there have been significant changes in the management of *BRCA*1/2 mutation carriers since the recruitment period of this study, including the exploration in trials of systemic therapies that exploit BRCA-null tumours including platinums and PARP inhibitors. The association of *BRCA* mutations with improved breast

cancer early outcomes in TNBC patients has the potential to influence early clinical trial results. As advanced genomic investigations increasingly become a part of routine oncological care, a significant proportion of breast cancer patients now learn their *BRCA* mutation status close to the time of diagnosis. In many cancer centres, immediate or post-chemotherapy bilateral mastectomy has become an almost routine recommendation for *BRCA1* and *BRCA2* mutation carriers regardless of the size or focality of the presenting tumour. In the longer term, for good prognosis breast cancers in particular, our data suggest that bilateral mastectomy and bilateral salpingo-oophorectomy would be expected to reduce deaths. Clinicians need to consider short and long term risks and benefits in discussing risk reducing bilateral mastectomy with patients. The number of patients in our TNBC group who had immediate bilateral mastectomy is small but our analysis suggests it is unlikely that the early bilateral mastectomy is accounting for the early survival advantage seen in the BRCA carriers with TNBC. With modern imaging using MRI breast screening, we conclude that patients who choose to delay additional surgery for one or two years until they are psychologically and physically recovered from their cancer treatment, can be reassured that this choice is unlikely to lead to any significant survival disadvantage. The importance of appropriately timed risk reducing bilateral salpingo-oophorectomy, for *BRCA*1 mutations carriers in particular, is clear but should take plans for further pregnancy into account. Furthermore, risk reducing bilateral salpingo-oophorectomy in very young women will have negative health consequences as a result of oestrogen deprivation from an early age.

The strengths of the POSH study include the large cohort size, low levels of missing data and young onset leading to a large number of *BRCA*1 and *BRCA*2 mutation carriers and high event rate ensuring that the study was well powered for the main outcome analysis. Our study minimises many of the biases in other studies by recruiting patients within the first year after diagnosis from oncology clinics nationally to minimise survival and selection bias and establishing BRCA carrier status for all patients included in the analysis. POSH participants recruited from England represented 23% of the available population during the recruitment period and comparison with cancer registry data has

confirmed that the POSH cohort is representative of the wider population.[16] Comprehensive details of pathology have enabled us to perform a separate analysis of outcome in patients with TNBC tumours; this is a unique contribution to this field. We have previously reported the significant and independent prognostic impacts of obesity and ethnicity on long-term outcomes in this young patient group; and this study is the only prospective study to date to include these host factors in multi-variable analyses.[21, 22]

Limitations of this study include the non-universal use of MLPA; we therefore cannot exclude the possibility that some structural variants have not been identified. However, even clinical diagnostic mutation testing is not 100% sensitive due to occult mutations not amenable to current methods (e.g. deep intronic splice variants); the investigation of *BRCA*1/2 gene sequences in this cohort was more comprehensive than in most other publications. All participants were tested for *TP53* mutations and carriers were excluded from this analysis because of the high risk of non-breast malignancies. We acknowledge that other breast cancer susceptibility gene variants have not been excluded; however these are expected to be very low in frequency and/or low penetrance and there is no evidence they specifically affect prognosis. We have outcome data from national flagging up to a median of 8·2 years. The treatment given reflects modern oncological practice with almost 90% of patients receiving neo/adjuvant chemotherapy, in over 95% of cases this was an anthracycline or anthracycline/taxane combination regimen.

Other limitations include restriction of the main cohort of the POSH study to patients aged 40 years or under at the time of diagnosis in order to enrich for *BRCA* mutation carriers. It is possible that observations in young onset breast cancer patients may not translate to older ages at diagnosis. Progesterone receptor testing was not performed routinely in many UK centres during the period of recruitment and supplementary data were derived from TMAs rather than full tumour sections. The relevance of TNBC in terms of biology and treatment has only become apparent since the POSH study was designed so the study is was not powered for this as the primary outcome, perhaps making it more noteworthy that the main difference in outcomes between carriers and non-carriers

was in this sub-group. Recommendations for adjuvant treatment in the UK changed over the course of recruitment with taxanes being recommended for node positive disease from 2006 and adjuvant trastuzumab for HER2 positive breast cancer routinely available only from 2006. Although we specifically collected information at 5 years about risk reducing surgery, we cannot exclude the possibility that risk reducing mastectomy and oophorectomy may have been performed at different hospitals from the recruiting cancer centre, (eg. at specialist plastic surgery or gynaecological units).

**Conclusions**

This study confirms that patients diagnosed with invasive breast cancer aged 18-40 years have a high breast cancer specific mortality and a high proportion of *BRCA*1 and *BRCA*2 gene carriers. We found no clear evidence that either *BRCA*1 or *BRCA*2 germline mutations significantly influence breast cancer prognosis after adjusting for known prognostic factors. Decisions about timing of risk reducing surgery should take into account primary tumour prognosis and patient preference. *BRCA* carriers presenting with TNBC may have an improved survival during the first few years after diagnosis compared to non-carriers although immediate bilateral mastectomy does not account for this advantage. Finally, analysis of early outcome data from trials exploring *BRCA*-deficient tumour treatment in TNBC should be interpreted with caution in view of the likely early survival advantage for BRCA carriers.

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**Declaration of Interests**

ERC declares honoraria from Roche, and RIC declares honoraria from GSK and Pfizer, DME declares honoraria from Astra Zeneca and Pierre Fabre. The other authors declared no conflicts of interest.

**Contributions of Authors**

The study was conceived and designed by DME, PS and DGA, planned and executed by DME, DGA, PS, DGE, AMT, PP, J LJ, HH, SL, RE, AH, FG, SH. Data acquisition, management and curation was performed by SG, LTD, ERC, TCM, WJT, RIC, SG-H, BE, LS and DME. LJ was responsible for central pathology review and AD and DFE supervised final research DNA sequencing. The statistical analysis plan was prepared by TM, DGA, DME, ERC and RIC. TM performed the statistical analysis and prepared figures. DME, ERC, TM, DGA and RIC interpreted the data and ERC, TM and DME wrote the manuscript. All authors critically reviewed iterations of the manuscript and approved the final draft for submission. ERC and TM are joint first authors.

## TABLES

**Table 1 – Patient and clinicopathological information for all patients (analysis population)**

| Characteristic | All patients (n=2733) | *BRCA*1+ (n=201) | *BRCA*2+ (n=137) | *BRCA*+ (n=338) | *BRCA*- (n=2395) | p-value† |
|---|---|---|---|---|---|---|
| Age at diagnosis, in years | | | | | | BRCA+ vs BRCA- |
| Median | 36 | 35 | 37 | 36 | 37 | p<0·0001 |
| Range | 18 to 40, | 22 to 40, | 21 to 40, | 21 to 40, | 18 to 40, | BRCA1+ vs BRCA2+ |
| IQR | 34 to 38 | 32 to 38 | 33 to 38 | 32 to 38 | 34 to 39 | p=0·014 |
| Missing | 0 | 0 | 0 | 0 | 0 | |
| Body Mass Index | | | | | | BRCA+ vs BRCA- |
| BMI<25kg/m$^2$ | 1427 (54·2%) | 114 (59·4%) | 70 (52·6%) | 184 (56·6%) | 1243 (53·9%) | p=0·48 |
| 25kg/m$^2$≤BMI<30kg/m$^2$ | 714 (27·1%) | 47 (24·5%) | 41 (30·8%) | 88 (27·1%) | 626 (27·1%) | BRCA1+ vs BRCA2+ |
| BMI≥30kg/m$^2$ | 491 (18·7%) | 31 (16·1%) | 22 (16·5%) | 53 (16·3%) | 438 (19·0%) | p=0·40 |
| Total | 2632 (100%) | 192 (100%) | 133 (100%) | 325 (100%) | 2307 (100%) | |
| Missing | 101 (3·7%) | 9 (4·5%) | 4 (2·9%) | 13 (3·8%) | 88 (3·7%) | |
| Ethnicity | | | | | | BRCA+ vs BRCA- |
| Caucasian/white | 2494 (92·4%) | 178 (90·8%) | 122 (91·0%) | 300 (90·9%) | 2194 (92·7%) | p=0·28 |
| Black | 103 (3·8%) | 10 (5·1%) | 6 (4·5%) | 16 (4·8%) | 87 (3·7%) | BRCA1+ vs BRCA2+ |
| Asian | 80 (3·0%) | 5 (2·6%) | 4 (3·0%) | 9 (2·7%) | 71 (3·0%) | p=0·99 |
| Other | 21 (0·8%) | 3 (1·5%) | 2 (1·5%) | 5 (1·5%) | 16 (0·7%) | |
| Total | 2698 (100%) | 196 (100%) | 134 (100%) | 330 (100%) | 2368 (100%) | |
| Missing | 35 (1·3%) | 5 (2·5%) | 3 (2·2%) | 8 (2·4%) | 27 (1·1%) | |
| Histological Grade | | | | | | BRCA+ vs BRCA- |
| 1 | 156 (5·9%) | 2 (1·0%) | 0 | 2 (0·6%) | 154 (6·6%) | p<0·0001 |

| Characteristic | All patients (n=2733) | *BRCA*1+ (n=201) | *BRCA*2+ (n=137) | *BRCA*+ (n=338) | *BRCA*- (n=2395) | p-value† |
|---|---|---|---|---|---|---|
| 2 | 904 (34·0%) | 16 (8·1%) | 40 (31·0%) | 56 (17·2%) | 848 (36·4%) | BRCA1+ vs BRCA2+ |
| 3 | 1598 (60·1%) | 179 (90·9%) | 89 (69·0%) | 268 (82·2%) | 1330 (57·0%) | p<0·0001 |
| Total | 2658 (100%) | 197 (100%) | 129 (100%) | 326 (100%) | 2332 (100%) | |
| Missing/not graded | 75 (2·7%) | 4 (2·0%) | 8 (5·8%) | 12 (3·6%) | 63 (2·6%) | |
| ER status | | | | | | BRCA+ vs BRCA- |
| Negative | 908 (33·4%) | 151 (75·5%) | 21 (15·4%) | 172 (51·2%) | 736 (30·9%) | p<0·0001 |
| Positive | 1811 (66·6%) | 49 (24·5%) | 115 (84·6%) | 164 (48·8%) | 1647 (69·1%) | BRCA1+ vs BRCA2+ |
| Total | 2719 (100%) | 200 (100%) | 136 (100%) | 336 (100%) | 2383 (100%) | p<0·0001 |
| Missing | 14 (0·5%) | 1 (0·5%) | 1 (0·7%) | 2 (0·6%) | 12 (0·5%) | |
| HER2 status | | | | | | BRCA+ vs BRCA- |
| Negative | 1763 (73·1%) | 164 (93·2%) | 111 (88·8%) | 275 (91·4%) | 1488 (70·5%) | p<0·0001 |
| Positive | 649 (26·9%) | 12 (6·8%) | 14 (11·2%) | 26 (8·6%) | 623 (29·5%) | BRCA1+ vs BRCA2+ |
| Total | 2412 (100%) | 176 (100%) | 125 (100%) | 301 (100%) | 2111 (100%) | p=0·18 |
| Missing | 321 (11·7%) | 25 (12·4%) | 12 (8·8%) | 37 (10·9%) | 284 (11·9%) | |
| PR status | | | | | | BRCA+ vs BRCA- |
| Negative | 951 (43·1%) | 144 (84·2%) | 23 (21·5%) | 167 (60·1%) | 784 (40·6%) | p<0·0001 |
| Positive | 1257 (56·9%) | 27 (15·8%) | 84 (78·5%) | 111 (39·9%) | 1146 (59·4%) | BRCA1+ vs BRCA2+ |
| Total | 2208 (100%) | 171 (100%) | 107 (100%) | 278 (100%) | 1930 (100%) | p<0·0001 |
| Missing | 525 (19·2%) | 30 (14·9%) | 30 (21·9%) | 60 (17·8%) | 465 (19·4%) | |
| *TNBC status | | | | | | BRCA+ vs BRCA- |
| Not TNBC | 2175 (79·6%) | 78 (38·8%) | 124 (90·5%) | 202 (59·8%) | 1973 (82·4%) | p<0·0001 |
| TNBC | 558 (20·4%) | 123 (61·2%) | 13 (9·5%) | 136 (40·2%) | 422 (17·6%) | BRCA1+ vs BRCA2+ |
| Total | 2733 (100%) | 201 (100%) | 137 (100%) | 338 (100%) | 2395 (100%) | p<0·0001 |

| Characteristic | All patients (n=2733) | *BRCA*1+ (n=201) | *BRCA*2+ (n=137) | *BRCA*+ (n=338) | *BRCA*- (n=2395) | p-value† |
|---|---|---|---|---|---|---|
| Missing | 0 | 0 | 0 | 0 | 0 | |
| Maximum tumour size (invasive), in mm | | | | | | BRCA+ vs BRCA- |
| Median | 22 | 21 | 25 | 22 | 22 | p=0·97 |
| Range | 0 to 170, | 1 to 140, | ·5 to 92, | ·5 to 140, | 0 to 170, | BRCA1+ vs BRCA2+ |
| IQR | 15 to 33 | 15 to 30 | 16 to 32 | 15 to 31 | 15 to 34 | p=0·060 |
| Missing | 156 (5·7%) | 10 (5·0%) | 14 (10·2%) | 24 (7·1%) | 132 (5·5%) | |
| Pathological N stage | | | | | | BRCA+ vs BRCA- |
| N0 | 1304 (48·4%) | 129 (64·2%) | 55 (40·7%) | 184 (54·8%) | 1120 (47·5%) | p=0·013 |
| N1 | 1388 (51·6%) | 72 (35·8%) | 80 (59·3%) | 152 (45·2%) | 1236 (52·5%) | BRCA1+ vs BRCA2+ |
| 1 to 3 | 899 (33·4%) | 43 (21·4%) | 51 (37·8%) | 94 (28·0%) | 805 (34·2%) | p<0·0001 |
| 4 to 9 | 330 (12·3%) | 14 (7·0%) | 19 (14·1%) | 33 (9·8%) | 297 (12·6%) | BRCA+ vs BRCA- |
| 10+ | 159 (5·9%) | 15 (7·5%) | 10 (7·4%) | 25 (7·4%) | 134 (5·7%) | p=0·019 |
| Total | 2692 (100%) | 201 (100%) | 135 (100%) | 336 (100%) | 2356 (100%) | BRCA1+ vs BRCA2+ |
| Missing | 41 (1·5%) | 0 | 2 (1·5%) | 2 (0·6%) | 39 (1·6%) | p=0·00017 |
| Lymphovascular invasion | | | | | | BRCA+ vs BRCA- |
| Absent | 1327 (52·3%) | 116 (61·1%) | 58 (46·8%) | 174 (55·4%) | 1153 (51·8%) | p=0·23 |
| Present | 1212 (47·7%) | 74 (38·9%) | 66 (53·2%) | 140 (44·6%) | 1072 (48·2%) | BRCA1+ vs BRCA2+ |
| Total | 2539 (100%) | 190 (100%) | 124 (100%) | 314 (100%) | 2225 (100%) | p=0·013 |
| Missing | 194 (7·1%) | 11 (5·5%) | 13 (9·5%) | 24 (7·1%) | 170 (7·1%) | |
| Chemotherapy timing | | | | | | BRCA+ vs BRCA- |
| None | 294 (10·8%) | 9 (4·5%) | 11 (8·0%) | 20 (5·9%) | 274 (11·4%) | p=0·0058 |

| **Characteristic** | **All patients (n=2733)** | ***BRCA*1+ (n=201)** | ***BRCA*2+ (n=137)** | ***BRCA*+ (n=338)** | ***BRCA*- (n=2395)** | **p-value†** |
|---|---|---|---|---|---|---|
| Adjuvant | 2027 (74·2%) | 171 (85·1%) | 99 (72·3%) | 270 (79·9%) | 1757 (73·4%) | BRCA1+ vs BRCA2+ |
| Neoadjuvant | 412 (15·1%) | 21 (10·4%) | 27 (19·7%) | 48 (14·2%) | 364 (15·2%) | p=0·016 |
| Total | 2733 (100%) | 201 (100%) | 137 (100%) | 338 (100%) | 2395 (100%) | |
| Missing | 0 | 0 | 0 | 0 | 0 | |
| Surgical Type | | | | | | BRCA+ vs BRCA- |
| BCS | 1337 (48·9%) | 106 (52·7%) | 43 (31·4%) | 149 (44·1%) | 1188 (49·6%) | p=0·30 |
| Mastectomy | 1373 (50·2%) | 94 (46·8%) | 92 (67·2%) | 186 (55·0%) | 1187 (49·6%) | BRCA1+ vs BRCA2+ |
| Nodal surgery only | 7 (0·3%) | 1 (0·5%) | 0 | 1 (0·3%) | 6 (0·3%) | p=0·00040 |
| None | 16 (0·6%) | 0 | 2 (1·5%) | 2 (0·6%) | 14 (0·6%) | |
| Total | 2733 (100%) | 201 (100%) | 137 (100%) | 338 (100%) | 2395 (100%) | |
| Missing | 0 | 0 | 0 | 0 | 0 | |
| Chemotherapy regimen | | | | | | BRCA+ vs BRCA- |
| None | 294 (10·8%) | 9 (4·5%) | 11 (8·0%) | 20 (5·9%) | 274 (11·4%) | p=0·015 |
| Anthracyclines | 1760 (64·4%) | 145 (72·1%) | 89 (65·0%) | 234 (69·2%) | 1526 (63·7%) | BRCA1+ vs BRCA2+ |
| Anthracyclines & Taxanes | 635 (23·2%) | 45 (22·4%) | 34 (24·8%) | 79 (23·4%) | 556 (23·2%) | p=0·38 |
| Taxanes | 24 (0·9%) | 0 | 1 (0·7%) | 1 (0·3%) | 23 (1·0%) | |
| Other (includes CMF) | 20 (0·7%) | 2 (1·0%) | 2 (1·5%) | 4 (1·2%) | 16 (0·7%) | |
| Total | 2733 (100%) | 201 (100%) | 137 (100%) | 338 (100%) | 2395 (100%) | |
| Missing | 0 | 0 | 0 | 0 | 0 | |

IQR=Inter-quartile range, ER=Oestrogen Receptor, HER2=Human Epidermal growth factor Receptor 2, PR=Progesterone Receptor, BCS=Breast Conserving Surgery.

†Test between *BRCA*+ vs. *BRCA*- patients, and *BRCA*1+ vs. *BRCA*2+ patients (excluding patients with both *BRCA*1 and *BRCA*2). Mann-Whitney tests used for continuous variables and Pearson $\chi^2$-tests for categorical variables, carried out on patients with complete data.

* TNBC= triple negative breast cancer, defined as ER negative, HER2 negative and PR negative or unknown.

**Table 2 – Patient and clinicopathological information for TNBC patients (TNBC population)**

| Characteristic | All patients (n=558) | BRCA1+ (n=123) | BRCA2+ (n=13) | BRCA+ (n=136) | BRCA- (n=422) | p-value† |
|---|---|---|---|---|---|---|
| Age at diagnosis, in years | | | | | | BRCA+ vs BRCA- |
| Median | 36 | 34 | 33 | 34 | 36 | p=0·00056 |
| Range | 19 to 40, | 22 to 40, | 30 to 40, | 22 to 40, | 19 to 40, | BRCA1+ vs BRCA2+ |
| IQR | 33 to 38 | 32 to 37 | 32 to 38 | 32 to 37 | 33 to 38 | p=0·79 |
| Missing | 0 | 0 | 0 | 0 | 0 | |
| Body Mass Index | | | | | | BRCA+ vs BRCA- |
| BMI<25kg/m$^2$ | 274 (50·2%) | 67 (56·3%) | 5 (38·5%) | 72 (54·5%) | 202 (48·8%) | p=0·26 |
| 25kg/m$^2$≤BMI<30kg/m$^2$ | 149 (27·3%) | 32 (26·9%) | 5 (38·5%) | 37 (28·0%) | 112 (27·1%) | BRCA1+ vs BRCA2+ |
| BMI≥30kg/m$^2$ | 123 (22·5%) | 20 (16·8%) | 3 (23·1%) | 23 (17·4%) | 100 (24·2%) | p=0·47 |
| Total | 546 (100%) | 119 (100%) | 13 (100%) | 132 (100%) | 414 (100%) | |
| Missing | 12 (2·2%) | 4 (3·3%) | 0 | 4 (2·9%) | 8 (1·9%) | |
| Ethnicity | | | | | | BRCA+ vs BRCA- |
| Caucasian/white | 500 (90·9%) | 110 (90·2%) | 9 (69·2%) | 119 (88·1%) | 381 (91·8%) | p=0·52 |
| Black | 26 (4·7%) | 7 (5·7%) | 2 (15·4%) | 9 (6·7%) | 17 (4·1%) | BRCA1+ vs BRCA2+ |
| Asian | 19 (3·5%) | 3 (2·5%) | 2 (15·4%) | 5 (3·7%) | 14 (3·4%) | p=0·052 |
| Other | 5 (0·9%) | 2 (1·6%) | 0 | 2 (1·5%) | 3 (0·7%) | |
| Total | 550 (100%) | 122 (100%) | 13 (100%) | 135 (100%) | 415 (100%) | |
| Missing | 8 (1·4%) | 1 (0·8%) | 0 | 1 (0·7%) | 7 (1·7%) | |
| Histological Grade | | | | | | BRCA+ vs BRCA- |
| 1 | 3 (0·6%) | 0 | 0 | 0 | 3 (0·7%) | p=0·49 |

| Characteristic | All patients (n=558) | BRCA1+ (n=123) | BRCA2+ (n=13) | BRCA+ (n=136) | BRCA- (n=422) | p-value† |
|---|---|---|---|---|---|---|
| 2 | 30 (5·5%) | 6 (4·9%) | 0 | 6 (4·4%) | 24 (5·9%) | BRCA1+ vs BRCA2+ |
| 3 | 508 (93·9%) | 116 (95·1%) | 13 (100·0%) | 129 (95·6%) | 379 (93·3%) | p=0·41 |
| Total | 541 (100%) | 122 (100%) | 13 (100%) | 135 (100%) | 406 (100%) | |
| Missing/not graded | 17 (3·0%) | 1 (0·8%) | 0 | 1 (0·7%) | 16 (3·8%) | |
| Maximum tumour size (invasive), in mm | | | | | | BRCA+ vs BRCA- |
| Median | 22 | 20·75 | 23·25 | 21 | 23 | p=0·17 |
| Range | 1 to 160, | 4 to 140, | 15 to 30, | 4 to 140, | 1 to 160, | BRCA1+ vs BRCA2+ |
| IQR | 15 to 31 | 15 to 30 | 16 to 30 | 15 to 30 | 15 to 32 | p=0·72 |
| Missing | 35 (6·3%) | 5 (4·1%) | 3 (23·1%) | 8 (5·9%) | 27 (6·4%) | |
| Pathological N stage | | | | | | BRCA+ vs BRCA- |
| N0 | 341 (61·8%) | 80 (65·0%) | 7 (58·3%) | 87 (64·4%) | 254 (60·9%) | p=0·46 |
| N1 | 211 (38·2%) | 43 (35·0%) | 5 (41·7%) | 48 (35·6%) | 163 (39·1%) | BRCA1+ vs BRCA2+ |
| 1 to 3 | 141 (25·5%) | 26 (21·1%) | 4 (33·3%) | 30 (22·2%) | 111 (26·6%) | p=0·64 |
| 4 to 9 | 45 (8·2%) | 7 (5·7%) | 0 | 7 (5·2%) | 38 (9·1%) | BRCA+ vs BRCA- |
| 10+ | 25 (4·5%) | 10 (8·1%) | 1 (8·3%) | 11 (8·1%) | 14 (3·4%) | p=0·044 |
| Total | 552 (100%) | 123 (100%) | 12 (100%) | 135 (100%) | 417 (100%) | BRCA1+ vs BRCA2+ |
| Missing | 6 (1·1%) | 0 | 1 (7·7%) | 1 (0·7%) | 5 (1·2%) | p=0·68 |
| Lymphovascular invasion | | | | | | BRCA+ vs BRCA- |
| Absent | 312 (60·3%) | 71 (61·2%) | 4 (40·0%) | 75 (59·5%) | 237 (60·6%) | p=0·83 |
| Present | 205 (39·7%) | 45 (38·8%) | 6 (60·0%) | 51 (40·5%) | 154 (39·4%) | BRCA1+ vs BRCA2+ |
| Total | 517 (100%) | 116 (100%) | 10 (100%) | 126 (100%) | 391 (100%) | p=0·19 |

| Characteristic | All patients (n=558) | BRCA1+ (n=123) | BRCA2+ (n=13) | BRCA+ (n=136) | BRCA- (n=422) | p-value† |
|---|---|---|---|---|---|---|
| Missing | 41 (7·3%) | 7 (5·7%) | 3 (23·1%) | 10 (7·4%) | 31 (7·3%) | |
| Chemotherapy timing | | | | | | BRCA+ vs BRCA- |
| None | 13 (2·3%) | 3 (2·4%) | 0 | 3 (2·2%) | 10 (2·4%) | p=0·17 |
| Adjuvant | 450 (80·6%) | 108 (87·8%) | 9 (69·2%) | 117 (86·0%) | 333 (78·9%) | BRCA1+ vs BRCA2+ |
| Neoadjuvant | 95 (17·0%) | 12 (9·8%) | 4 (30·8%) | 16 (11·8%) | 79 (18·7%) | p=0·074 |
| Total | 558 (100%) | 123 (100%) | 13 (100%) | 136 (100%) | 422 (100%) | |
| Missing | 0 | 0 | 0 | 0 | 0 | |
| Surgical Type | | | | | | BRCA+ vs BRCA- |
| BCS | 331 (59·3%) | 69 (56·1%) | 5 (38·5%) | 74 (54·4%) | 257 (60·9%) | p=0·19 |
| Mastectomy | 223 (40·0%) | 53 (43·1%) | 7 (53·8%) | 60 (44·1%) | 163 (38·6%) | BRCA1+ vs BRCA2+ |
| Nodal surgery only | 1 (0·2%) | 1 (0·8%) | 0 | 1 (0·7%) | 0 | p=0·014 |
| None | 3 (0·5%) | 0 | 1 (7·7%) | 1 (0·7%) | 2 (0·5%) | |
| Total | 558 (100%) | 123 (100%) | 13 (100%) | 136 (100%) | 422 (100%) | |
| Missing | 0 | 0 | 0 | 0 | 0 | |
| Chemotherapy regimen | | | | | | BRCA+ vs BRCA- |
| None | 13 (2·3%) | 3 (2·4%) | 0 | 3 (2·2%) | 10 (2·4%) | p=0·097 |
| Anthracyclines | 382 (68·5%) | 91 (74·0%) | 6 (46·2%) | 97 (71·3%) | 285 (67·5%) | BRCA1+ vs BRCA2+ |
| Anthracyclines & Taxanes | 159 (28·5%) | 27 (22·0%) | 7 (53·8%) | 34 (25·0%) | 125 (29·6%) | p=0·086 |
| Taxanes | 2 (0·4%) | 0 | 0 | 0 | 2 (0·5%) | |
| Other (includes CMF) | 2 (0·4%) | 2 (1·6%) | 0 | 2 (1·5%) | 0 | |
| Total | 558 (100%) | 123 (100%) | 13 (100%) | 136 (100%) | 422 (100%) | |
| Missing | 0 | 0 | 0 | 0 | 0 | |

IQR=Inter-quartile range, BCS=Breast Conserving Surgery.

†Test between *BRCA*+ vs. *BRCA*- patients, and *BRCA*1+ vs. *BRCA*2+ patients (excluding patients with both *BRCA*1 and *BRCA*2). Mann-Whitney tests used for continuous variables and Pearson $\chi^2$-tests for categorical variables, carried out on patients with complete data..

# FIGURES

**Figure 1 – Overall Survival by *BRCA* status for all patients (analysis population)**

Kaplan-Meier plot by *BRCA*1 and/or 2 status (*BRCA*+/-) for Overall Survival (OS) (Panel A); and Forest Plot of corresponding univariable and multivariable hazard ratios by *BRCA*+/- status for Overall Survival (Panel B). In Panel B, multivariable analysis is adjusted for age, body mass index, grade, tumour size, HER2 status, ER status, ethnicity and use of taxane chemotherapy.

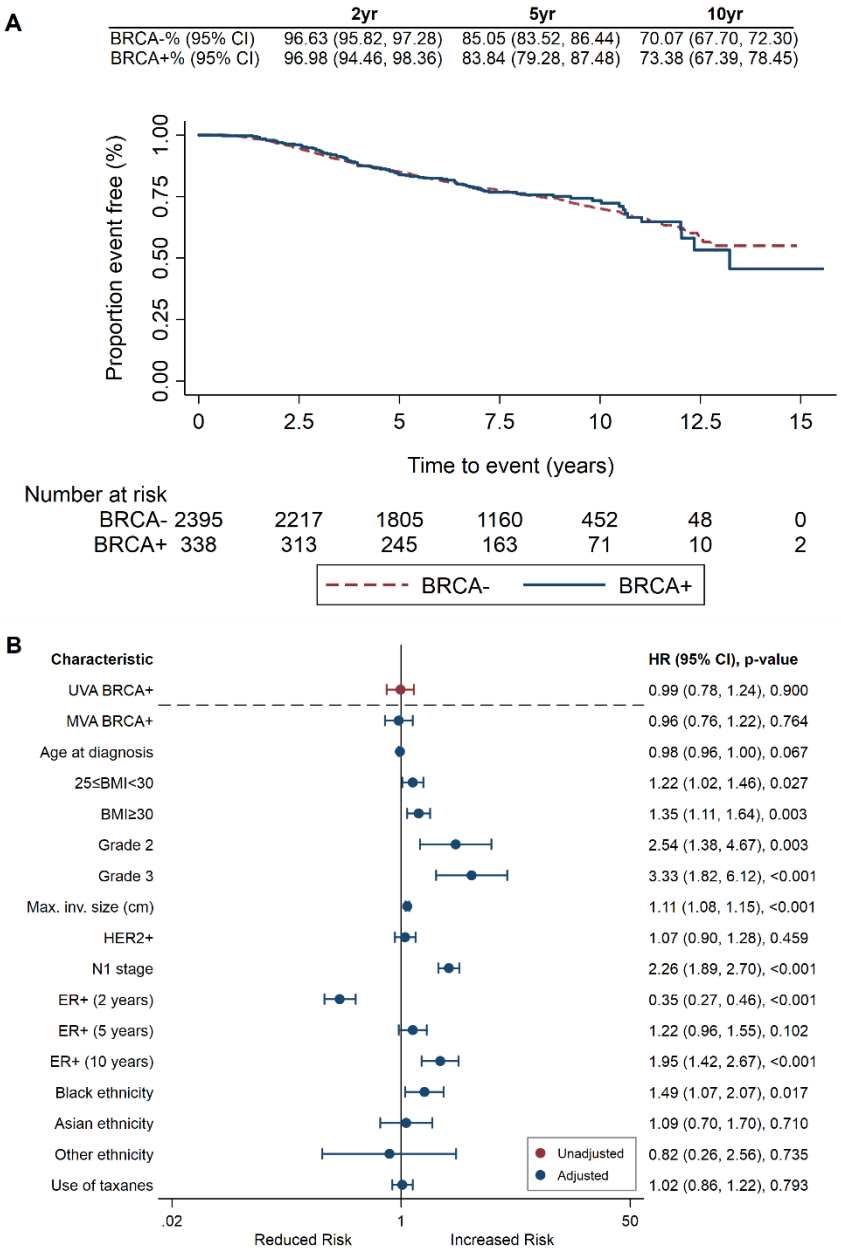


**Figure 2 – Time-varying effects of *BRCA* status on Overall Survival for all TNBC patients (TNBC population)**

Time-varying hazard rates by *BRCA*1 and/or 2 status (*BRCA*+/-) for Overall Survival (OS) (Panel A); and corresponding time-varying hazard ratio for Overall Survival (Panel B).

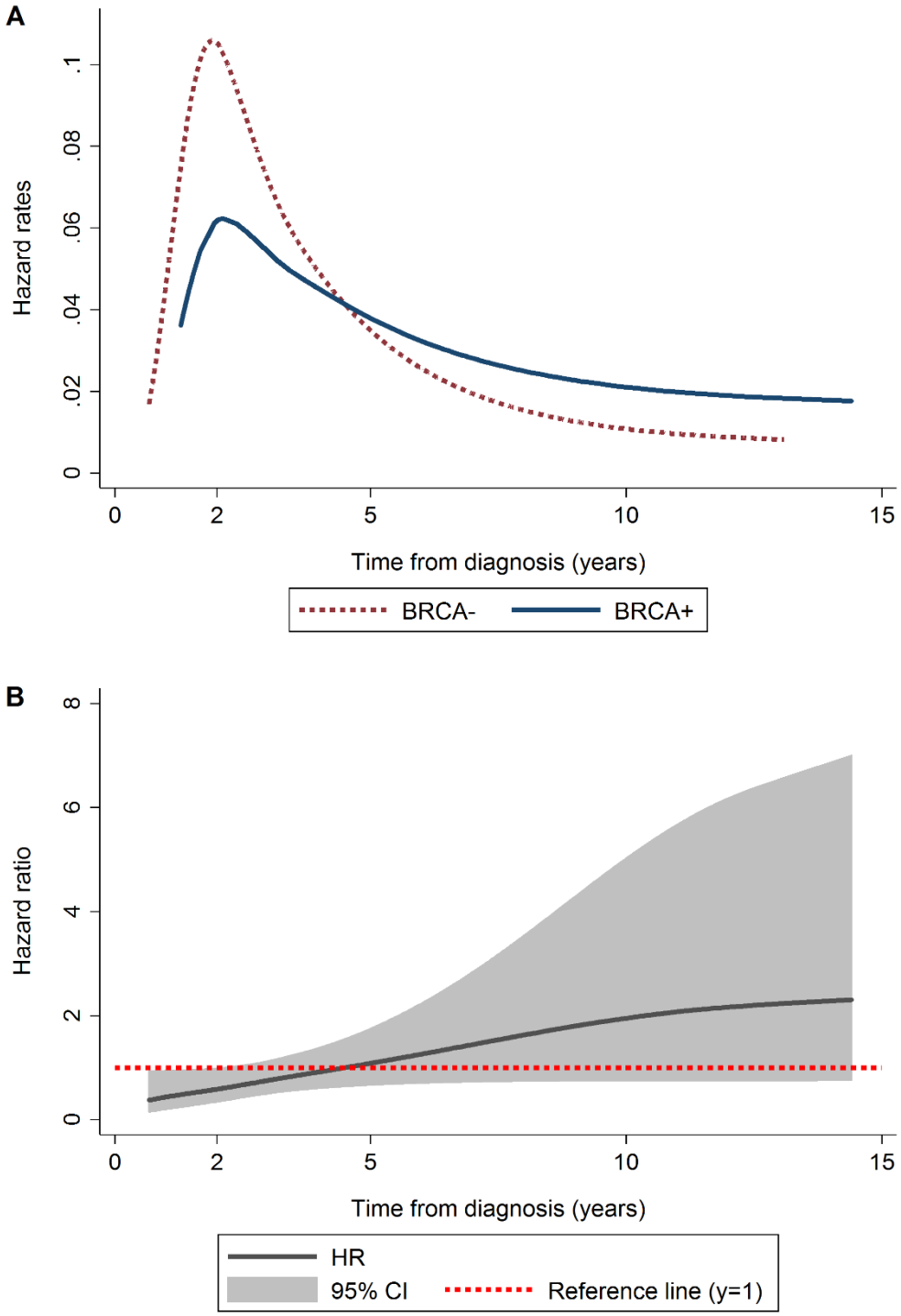
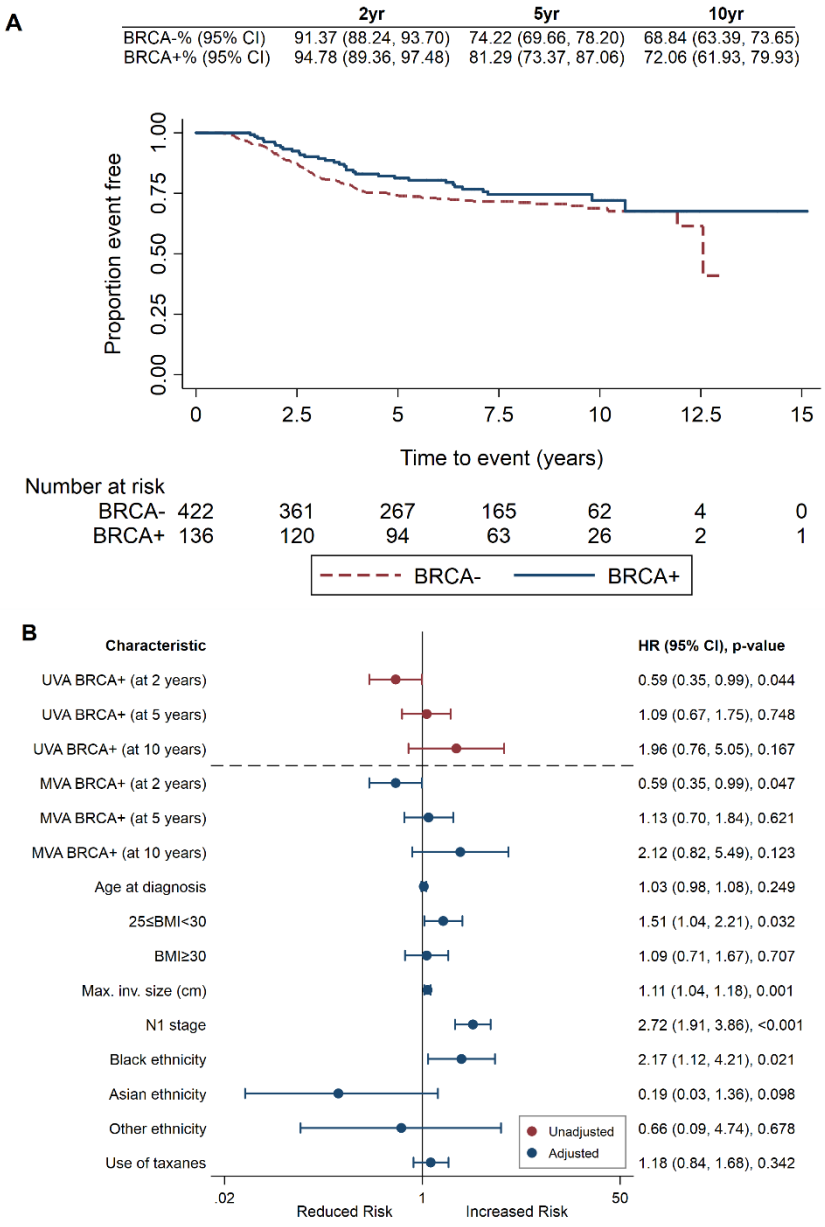


Figure 3 – Overall Survival by *BRCA* status for all TNBC patients (TNBC population)

Kaplan-Meier plot by *BRCA*1 and/or 2 status (*BRCA*+/-) for Overall Survival (OS) (Panel A); and Forest Plot of corresponding univariable and multivariable hazard ratios by *BRCA*+/- status for Overall Survival (Panel B). In Panel B, multivariable analysis is adjusted for age, body mass index, tumour size, ethnicity and use of taxane chemotherapy.



Appendix

**Appendix Table 1: Recruitment by active sites**

List of recruitment number by all active sites in the reported cohort.

**Appendix Table 2: List of BRCA1 and BRCA2 mutation annotation**

List of 338 pathogenic BRCA1 and BRCA2 variants included in the BRCA+ group

**Appendix Table 3: Cause of death breakdown by BRCA status (analysis population who died)**

List of all causes of death in the reported cohort.

**Appendix Table 4: Multivariable Analyses - Complete-Case Results (analysis population)**

Breakdown of compete-case results for each multivariable analysis carried out on the analysis population.

**Appendix Table 5: Multivariable Analyses - Complete-Case Results (TNBC population)**

Breakdown of compete-case results for each multivariable analysis carried out on the TNBC population.

**Appendix Figure 1 – Flow diagram of the POSH cohort**

Flow diagram of the POSH cohort.

**Appendix Figure 2 – Distant Disease Free Survival by *BRCA* status for all patients (analysis population)**

Kaplan-Meier plot by *BRCA*1 and/or 2 status (*BRCA*+/-) for Distant Disease Free Survival (OS) (Panel A); and Forest Plot of corresponding univariable and multivariable hazard ratios by *BRCA*+/- status for Distant Disease Free (Panel B). In Panel B, multivariable analysis is adjusted for age, body mass index, grade, tumour size, HER2 status, ER status, ethnicity and use of taxane chemotherapy.

**Appendix Figure 3 – Overall Survival by *BRCA1* status for all patients (analysis population)**

Kaplan-Meier plot by *BRCA1* status (*BRCA1*+/-) for Overall Survival (OS) (Panel A); and Forest Plot of corresponding univariable and multivariable hazard ratios by *BRCA1*+/- status for Overall Survival (Panel B). In

Panel B, multivariable analysis is adjusted for age, body mass index, grade, tumour size, HER2 status, ER status, ethnicity and use of taxane chemotherapy.

**Appendix Figure 4 – Overall Survival by *BRCA2* status for all patients (analysis population)**

Kaplan-Meier plot by *BRCA2* status (*BRCA2*+/-) for Overall Survival (OS) (Panel A); and Forest Plot of corresponding univariable and multivariable hazard ratios by *BRCA2*+/- status for Overall Survival (Panel B). In Panel B, multivariable analysis is adjusted for age, body mass index, grade, tumour size, HER2 status, ER status, ethnicity and use of taxane chemotherapy.

**Appendix Figure 5 – Distant Disease Free Survival by *BRCA* status for all TNBC patients (TNBC population)**

Kaplan-Meier plot by *BRCA*1 and/or 2 status (*BRCA*+/-) for Distant Disease Free Survival (OS) (Panel A); and Forest Plot of corresponding univariable and multivariable hazard ratios by *BRCA*+/- status for Distant Disease Free Survival (Panel B). In Panel B, multivariable analysis is adjusted for age, body mass index, tumour size, ethnicity and use of taxane chemotherapy.

**Appendix Figure 6 – Overall Survival by *BRCA* status for all patients, adjusting for time to blood draw (analysis population)**

Forest Plot of univariable and multivariable hazard ratios by *BRCA*+/- status for Overall Survival (OS), adjusting for time to blood draw. Multivariable analysis is also adjusted for age, body mass index, grade, tumour size, HER2 status, ER status, ethnicity and use of taxane chemotherapy.

**Appendix Figure 7 – Multivariable Analyses - Proportional hazards tests**

Proportional hazards (PH) test results for the main comparators for: (A) Overall Survival (OS) by BRCA status – analysis population (PH assumption met); (B) Distant disease free survival (DDFS) by BRCA status – analysis population (PH assumption met); (C) OS by BRCA1 status – analysis population (PH assumption met); (D) OS by BRCA2 status – analysis population (PH assumption met); (E) OS by BRCA status – TNBC population (PH assumption not met); (F) DDFS by BRCA status – TNBC

**Appendix Figure 8 – Overall Survival by *BRCA* status for TNBC patients not having immediate bilateral mastectomies (TNBC population, excluding patients not having immediate bilateral mastectomies)**

Forest Plot of univariable and multivariable hazard ratios by *BRCA*+/- status for Overall Survival (OS).

Multivariable analysis is adjusted for age, body mass index, tumour size, ethnicity and use of taxane chemotherapy.

**Appendix Figure 9 – Overall Survival by *BRCA* status for TNBC patients who did not develop a new primary breast or ovarian cancer (TNBC population, excluding patients who developed a new primary breast or ovarian cancer)**

Forest Plot of univariable and multivariable hazard ratios by *BRCA*+/- status for Overall Survival (OS).

Multivariable analysis is adjusted for age, body mass index, tumour size, ethnicity and use of taxane chemotherapy.

**Appendix Methods 1: BRCA1 and BRCA2 gene sequencing and variant calling**

Details of sequencing methodology and annotation of variants.

**Appendix Document 1: Statistical Analysis Plan**

Statistical analysis plan (SAP), approved on 10-May-2016, and formatted for Lancet Oncology Appendix.

**Appendix Document 2: STROBE Checklist**

Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist.

## REFERENCES


1. Anglian Breast Cancer Study Group. Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. *Br J Cancer*. 2000; **83**: 13018.
2. Malone KE, Daling JR, Doody DR, et al. Prevalence and predictors of BRCA1 and BRCA2 mutations in a population-based study of breast cancer in white and black American women ages 35 to 64 years. Cancer Research. 2006; **66**: 8297-308.
3. Anders CK, Hsu DS, Broadwater G, et al. Young age at diagnosis correlates with worse prognosis and defines a subset of breast cancers with shared patterns of gene expression. *J Clin Onco*l. 2008; **26**: 3324-30.
4. Turner NC and Tutt AN. Platinum chemotherapy for BRCA1-related breast cancer: do we need more evidence? *Breast Cancer Res*. 2012; **14**: 115.
5. Lakhani SR, Jacquemier J, Sloane JP, et al. Multifactorial analysis of differences between sporadic breast cancers and cancers involving BRCA1 and BRCA2 mutations. *J Natl Cancer Inst*. 1998 ;**90**: 1138–45.
6. Mavaddat N, Barrowdale D, Andrulis IL, et al. Pathology of breast and ovarian cancers among BRCA1 and BRCA2 mutation carriers: results from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). *Cancer Epidemiol Biomarkers Prev*. 2012; **21**: 134-47.
7. Byrski T, Gronwald J, Huzarski T, et al. Pathologic complete response rates in young women with BRCA1-positive breast cancers after neoadjuvant chemotherapy. *J Clin Oncol*. 2010; **14**: 375-9.
8. Rennert G, Bisland-Naggan S, Barnett-Griness O, et al. Clinical outcomes of breast cancer in carriers of BRCA1 and BRCA2 mutations. *N Engl J Med* 2007; **357**: 115-23.
9. Verhoog LC, Brekelmans CT, Seynaeve C, et, al. Survival and tumour characteristics of breast cancer patients with germline mutations of BRCA1. *Lancet*. 1998; **351**: 316-21.
10. Huzarski T, Byrski T, Gronwald J, et al. Ten year survival in patients with BRCA1-negative and BRCA1-positive breast cancer. *J Clin Oncol* 2013; **31**: 3191-6.
11. Bordeleau L, Panchal S, Goodwin P. Prognosis of BRCA-associated breast cancer: a summary of evidence. *BreastCancer Res Treat*. 2010; **119**: 13-24.
12. van den Broek AJ, Schmidt MK, van 't Veer LJ, Tollenaar RA, van Leeuwen FE. Worse breast cancer prognosis of BRCA1/BRCA2 mutation carriers: what's the evidence? A systematic review with meta-analysis. *PloS one*. 2015; **10**:e0120189.
13. Baretta Z, Mocellin S, Goldin E, Olopade OI, Huo D. Effect of BRCA germline mutations on breast cancer prognosis: A systematic review and meta-analysis. *Medicine (Baltimore).* 2016;**95**: e4975.
14. Schmidt MK, Van den Broek AJ, Tollenaar RA, et al. Breast cancer survival of BRCA1/2 mutation carriers in a hospital based cohort of young women. *JNCI*. 2017; **109**: djw329.
15. Eccles D, Gerty S, Simmonds P, Hammond V, Ennis S, Altman DG. Prospective study of Outcomes in Sporadic versus Hereditary breast cancer (POSH): study protocol. *BMC Cancer*. 2007; **7**: 160.
16. Copson E, Eccles B, Maishman T, et al. Prospective observational study of breast cancer treatment outcomes for UK women aged 18-40 years at diagnosis: the POSH study. *JNCI*. 2013; **105**: 978-88.
17. Antoniou AC, Cunningham AP, Peto J ED, et al. The BOADICEA model of genetic susceptibility to breast and ovarian cancers: updates and extensions. *Br J Cancer*. 2008; **98**: 1457-66.
18. National Institute of Clinical Excellence. Familial breast cancer: classification, care and managing breast cancer and related risks in people with a family history of breast cancer. 2013.

19. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of ObservationalStudies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet*. 2007; **370**: 1453–1457
20. Lambert PC and Royston P. Further development of flexible parametric models for survival analysis. *Stata J.* 2009; **9**: 265-90.
21. Copson E, Maishman T, Gerty S, et al. Ethnicity and outcome of young breast cancer patients in the United Kingdom: the POSH study. *Brit J Cancer*. 2014; **110**: 230-41.
22. Copson ER, Cutress RI, Maishman T, et al. Obesity and the outcome of young breast cancer patients in the UK: the POSH study. *Annals Oncol*. 2015; **26**: 101-12.
23. Goodwin PJ, Phillips KA, West DW, et al. Breast cancer prognosis in BRCA1 and BRCA2 mutation carriers: an International Prospective Breast Cancer Family Registry population-based cohort study. *J Clin Oncol*. 2012; **30**: 19-26.
24. Robson M, Chappuis PO, Satagopan J, et al. A combined analysis of outcome following breast cancer: differences in survival basd on BRCA1/2 mutation status and administration of adjuvant treatment. *BreastCancer Res*. 2004; **6**: R8-R17.
25. Stoppa-Lyonnet D, Ansquer Y, Dreyfus H, et al. Familial invasive breast cancers: worse outcome related to BRCA1 mutations. *J Clin Oncol* 2000; **18**: 4053-9.
26. Moller P, Evans DG, Reis MM, et al. Surveillance for familial breast cancer: Differences in outcome according to BRCA mutation status. *Int J Cancer*. 2007; **121**: 1017-20.
27. Maishman T, Cutress RI, Hernandez A, et al. Local Recurrence and Breast Oncological Surgery in Young Women With Breast Cancer: The POSH Observational Cohort Study. *Annals Surg.* 2017;266: 165-172.
28. Heemskerk-Gerritsen BA, Seynaeve C, Van Asperen CJ, et al. Breast cancer risk after salpingo-oophorectomy in healthy BRCA1/2 mutation carriers: revisiting the evidence for risk reduction. *JNCI*. 2015; **107**: djv033.
29. Bolton KL, Chenevix-Trench G, Goh C, et al. Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. *JAMA.* 2012; **307**: 382-90.
30. Candido Dos Reis FJ, Song H, Goode EL, et al. Germline mutation in BRCA1 or BRCA2 and ten-year survival for women diagnosed with epithelial ovarian cancer. *Clin Cancer Res*. 2015; **21**: 652-7.
31. Jiang T, Shi W, Wali VB, et al. Predictors of Chemosensitivity in Triple Negative Breast Cancer: An Integrated Genomic Analysis. *PLoS Med* . 2016; **13**: e1002193.